

Please replace the paragraph beginning on page 2, line 2, with the following rewritten paragraph:

---

A2 -- The fungus, *Aspergillus fumigatus* causes a wide spectrum of human and animal disorders such as allergic bronchopulmonary aspergillosis (ABPA), extrinsic allergic alveolitis, aspergilloma and invasive aspergillosis. An invasive form of aspergillosis is becoming increasingly important in immunosuppressed conditions due to environmental pollution, enhanced use of chemotherapeutic drugs and antibiotics etc. The most susceptible hosts are immunocompromised patients, such as cases with organ transplant, leukemia or acquired immunodeficiency syndrome (AIDS).--

---

Please replace the paragraph beginning on page 2, line 10, with the following rewritten paragraph:

---

A3 -- Currently available tests for identification of this fungus is based on tedious, time consuming, less sensitive methods such as microscopy, cultures, electrophoresis and immunodiffusion. Many clinical features of aspergillosis are similar to tuberculosis and most of the aspergillosis patients are put on antituberculous therapy. The microscopy of the specimen for identification of *Aspergillus* hyphae is not easy under field conditions and specimens from the patients in the early stages of disease are often negative in the direct mounts. Further, the fungal culture generally takes 3-4 weeks and is expensive as a routine diagnostic measure. The widely used skin testing for *Aspergillus* allergic patients lacks sensitivity and specificity as the mixture of allergens used for testing is not well characterised and needs standardisation. The steroid therapy used for allergic

A3  
cont

patients and chemotherapy for invasive patients are more beneficial when employed in the early stages of the disease. Consequent to these factors, many investigators recommend that early diagnosis of aspergillosis should be considered as a priority area of research and development.--

Please replace the paragraph beginning on page 3, line 9, with the following rewritten paragraph:

A4

-- Many investigators have identified protein allergens and antigens of *A. fumigatus* which have potential immunodiagnostic application. The N-terminal and internal amino acid sequences of many of these allergens/antigens have been published. The N-terminal amino acid sequences of some of these are presented in Table 1.--

Please replace the paragraph beginning on page 3, line 14, with the following rewritten paragraph:

--TABLE -1

*A. fumigatus* protein allergens/antigens identified by N-terminal amino acid sequences.

Investigators	Antigens and N-terminus
Teshima <u>et al</u> , 1993	55 kDa: ATPHEPVFFSWDAGAVTSFP (SEQ ID NO:8)
Kumar <u>et al</u> , 1993	65 kDa: AQNRQTLAKLLRYQSTKSG (SEQ ID NO:9)
Moser <u>et al</u> , 1992	18 kDa: ATWTCINQQLNPKNKWE (SEQ ID NO:10)
Arruda <u>et al</u> , 1992	18 kDa: ATWTCINQQLNPKNKWE (SEQ ID NO:10)
Banerjee <u>et al</u> , 1996	34 kDa: SARDEAGLNEAVELARHAK (SEQ ID NO:11)--

Please replace the paragraph beginning on page 6, line 18, with the following rewritten paragraph:

-- Accordingly, the present invention provides *Aspergillus fumigatus* peptides having an amino acid sequence selected from the immunodominant region delimited by aa 6-22 of the 18 kD allergen/antigen, the said peptides having the following sequences useful for immunodiagnosis of aspergillosis;

- A7
- Isoleucinyl-asparaginyl-glutamyl-glutamyl-leucyl-asparaginyl-prolyl-lysyl-threonyl-asparaginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysyl [INQQLNPKTNKWEDKRY] (aa 6-22) (SEQ ID NO:1)
  - Leucyl-asparaginyl-prolyl-lysyl-threonyl-asparaginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysyl-arginyl-tyrosine [LNPKTNKWEDK] (aa 10-20) (SEQ ID NO:2)
  - Isoleucyl-asparaginyl-gluamyl-glutamyl-leucyl-asparaginyl-prolyl-lysyl [INQQLNPK] (aa 6-14) (SEQ ID NO: 3)
  - Isoleucinyl-asparaginyl-glutamyl-glutamyl-leucyl-asparaginyl-prolyl-lysyl-threonyl-asparaginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysyl INQQLNPKTNKWEDK (aa 6-20) (SEQ ID NO: 4)
  - Threonyl-asparaginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysine [TNKWEDK] (aa 14-20) (SEQ ID NO: 5)
  - Lysyl-lysyl-leucyl-asparaginyl-prolyl-lysyl-threonyl-asparaginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysyl-lysyl-lysine (aa10-22) [LNPKTNKWEDKRY] (SEQ ID NO: 6)--

Please replace the paragraph beginning on page 7, line 22, with the following rewritten paragraph:

A8 --3. The protecting moiety from the alpha-amino group of the amino acid is removed later by hydrochloride acid/dioxane, trifluoroacetic acid, piperidine etc.--

Please replace the paragraph beginning on page 8, line 4, with the following rewritten paragraph:

A9 --5. The steps of coupling and deblocking are repeated with other suitable protected amino acids of the peptide sequence.--

Please replace the paragraph beginning on page 8, line 6, with the following rewritten paragraph:

A10 --6. After the completion of coupling of all the required amino acids of the peptide sequence, the peptide is cleaved from the resin by acid treatment followed by neutralisation and deblocking the protecting groups.--

Please replace the paragraph beginning on page 8, line 20, with the following rewritten paragraph:

A11 --(a) Lysyl-lysyl-leucyl-asparginyl-prolyl-lysyl-threonyl-asparginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysyl-lysine (KKLNPKTNKWEDKKK) (SED ID NO: 7),--

Please replace the paragraph beginning on page 8, line 22, with the following rewritten paragraph:

A12 --(b) Threonyl-asparginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysine, (TNKWEDK) (SEQ ID NO: 5),--

Please replace the paragraph beginning on page 9, line 1, with the following rewritten paragraph:

A13

--(c) Isoleucyl-asparaginyl-glutaminyl-glutaminyl-leucyl-  
asparaginyl-proly-lysine (INQQLNPK) (SEQ ID NO: 3),--

Please replace the paragraph beginning on page 9, line 3, with the  
following rewritten paragraph:

A14

--(d) Leucyl-asparaginyl-prolyl-lysyl-threonyl-asparaginyl-  
lysyl-tryptophanyl-glutamyl-aspartyl-lysyl-arginyl-tyrosine  
(LNPKTNKWEDKRY) (SEQ ID NO: 6), or--

Please replace the paragraph beginning on page 9, line 5, with the  
following rewritten paragraph:

A15

--(e) Isoleucinyl-asparaginyl-glutaminyl-glutaminyl-leucyl-  
asparaginyl-prolyl-lysyl-threonyl-asparaginyl-lysyl-  
tryptophanyl-glutamyl-aspartyl-lysyl (INQQLNPKTNKWEDK) (SEQ ID  
NO: 4).--

Please replace the paragraph beginning on page 9, line 16, with the  
following rewritten paragraph:

A16

-- In yet another embodiment of the invention deblocking  
agents used may be such as HCl/dioxane, TFA/CH<sub>2</sub>Cl<sub>2</sub> HBr /AcOH,  
formic acid etc.--

Please replace the paragraph beginning on page 10, line 1, with the  
following rewritten paragraph:

A17

-- In another embodiment of the invention hydrogenation agents  
used may be such as Pd/C, Palladium chloride, Rhodium/C, adams  
catalysts and palladium black etc.--

Please replace the paragraph beginning on page 10, line 4, with the  
following rewritten paragraph:

A18

-- The present invention further relates to a method for using any of the peptide sequences stated above and falling under the immunodominant region delimited by aa 6-22 for the diagnosis of aspergillosis which comprises; --

Please replace the paragraph beginning on page 10, line 7, with the following rewritten paragraph:

A19

-- a. collecting body fluid sample from a patient and separating the fluid from the cells,--

Please replace the paragraph beginning on page 10, line 12, with the following rewritten paragraph:

A20

-- d. incubating the antibodies obtained in step c with the mixture of allergens/antigens of *A. fumigatus* coated on the polystyrene ELISA plates,--

Please replace the paragraph beginning on page 11, line 1, with the following rewritten paragraph:

A21

-- Furthermore, the present invention relates to a method for using the peptide sequence as stated above and from aa 10-20 for the diagnosis of aspergillosis which further comprises;--

Please replace the paragraph beginning on page 11, line 4, with the following rewritten paragraph:

A22

-- a. collecting body fluid sample from a patient and separating the fluid from the cells,--

Please replace the paragraph beginning on page 11, line 20, with the following rewritten paragraph:

A23

--In an embodiment of the invention, the body fluid used may be selected from blood, serum, cerebrospinal fluid, pleural

A 23  
cont

fluids and saliva. --

Please replace the paragraph beginning on page 11, line 22, with the following rewritten paragraph:

A 24

--In another embodiment of the invention, *A. fumigatus* allergens/antigens used are either obtained commercially or prepared by known methods.--

Please replace the paragraph beginning on page 12, line 6, with the following rewritten paragraph:

A 25

-- In an embodiment of the invention, body fluid used may be selected from blood, serum, cerebrospinal fluid, pleural fluids and saliva.--

Please replace the paragraph beginning on page 12, line 8, with the following rewritten paragraph:

A 26

-- In another embodiment of the invention, the *A. fumigatus* allergens/antigens used are either obtained commercially or prepared by known methods.--

Please replace the paragraph beginning on page 13, line 23, with the following paragraph:

A 27

-- Figure 1 shows the sequence of isoleucinyl-asparaginyl-glutamyl-glutamyl-leucyl-asparaginyl-prolyl-lysyl-threonyl-asparaginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysyl [INQQLNPKTNKWEDKRY] (-aa 6-22) [SEQ ID NO: 1]--

Please replace the paragraph beginning on page 14, line 1, with the following rewritten paragraph:

A 28

-- Figure 2 shows the polypeptide sequence of Leucyl-asparaginyl-prolyl-lysyl-threonyl-asparaginyl-lysyl-

A28  
cont

tryptophanyl-glutamyl-aspartyl-lysyl-arginyl-tyrosine  
[LNPKTNKWEDK] (aa 10-20) [SEQ ID NO: 2]--

---

Please replace the paragraph beginning on page 14, line 4, with the following rewritten paragraph:

---

A29

--Figure 3 provides the sequence of Isoleucyl-asparaginyl-glutamyl-glutamyl-leucyl-asparaginyl-prolyl-lysyl [INQQLNPK] (aa 6-14) [SEQ ID NO: 3]--

---

Please replace the paragraph beginning on page 14, line 6, with the following rewritten paragraph:

---

A30

--Figure 4 provides the sequence of soleucinyl-asparaginyl-glutamyl-glutamyl-leucyl-asparaginyl-prolyl-lysyl-threonyl-asparaginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysyl INQQLNPKTNKWEDK (aa 6-20) [SEQ ID NO:4] --

---

Please replace the paragraph beginning on page 14, line 10, with the following rewritten paragraph:

---

A31

--Figure 5 provides the sequence of Threonyl-asparaginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysine [TNKWEDK] (aa 14-20) [SEQ ID NO:5]--

---

Please replace the paragraph beginning on page 14, line 12, with the following rewritten paragraph:

---

A32

--Figure 6 provides the sequence of Lysyl-lysyl-leucyl-asparaginyl-prolyl-lysyl-threonyl-asparaginyl-lysyl-tryptophanyl-glutamyl-aspartyl-lysyl-lysyl-lysine [LNPKTNKWEDKRY] (aa 10-22) [SEQ ID NO:6]--

---



Please replace the paragraph beginning on page 14, line 15, with the following rewritten paragraph:

A33 --Figure 7 of the accompanying drawings shows Fast Atom Bombardment Mass spectra for peptide (SEQ ID NO: 2)--

Please replace the paragraph beginning on page 14, line 17, with the following rewritten paragraph:

A34 --Figure 8 of the accompanying drawings shows Fast Atom Bombardment Mass spectra for peptide (SEQ ID NO: 3)--

Please replace the paragraph beginning on page 15, line 1, with the following rewritten paragraph:

A35 --Figure 9 of the accompanying drawings shows Fast Atom Bombardment Mass spectra for peptide (SEQ ID NO: 4) --

Please replace the paragraph beginning on page 15, line 3, with the following rewritten paragraph:

A36 --Figure 10 of the accompanying drawings shows Fast Atom Bombardment Mass spectra for peptide (SEQ ID NO: 5) --

Please replace the paragraph beginning on page 15, line 5, with the following rewritten paragraph:

A37 --Figure 11 of the accompanying drawings shows Fast Atom Bombardment Mass spectra for peptide (SEQ ID NO:6)--

Please replace the paragraph beginning on page 15, line 7, with the following rewritten paragraph:

A38 --Figure 12 of the accompanying drawings shows High performance liquid chromatography profile for peptide (SEQ ID NO:2)--

Please replace the paragraph beginning on page 15, line 9, with the following rewritten paragraph:

A39 --Figure 13 of the accompanying drawings shows High performance liquid chromatography profile for peptide (SEQ ID NO:3) --

Please replace the paragraph beginning on page 15, line 11 with the following rewritten paragraph:

A40 --Figure 14 of the accompanying drawings shows High performance liquid chromatography profile for peptide (SEQ ID NO:4) --

Please replace the paragraph beginning on page 15, line 13 with the following rewritten paragraph:

A41 --Figure 15 of the accompanying drawings shows High performance liquid chromatography profile for peptide (SEQ ID NO:5) --

Please replace the paragraph beginning on page 15, line 15 with the following rewritten paragraph:

A42 --Figure 16 of the accompanying drawings shows High performance liquid chromatography profile for peptide (SEQ ID NO:6) --

Please replace the paragraph beginning on page 15, line 18, with the following rewritten paragraph:

---

-- Identification of peptide epitopes in the 18 kD of A.

*fumigatus*

A43 An allergen/antigen with an apparent molecular weight of 18 kD was isolated, purified and characterised from the third week culture filtrate of *Aspergillus fumigatus* (strain 285, isolated from the sputum of an ABPA patient similar to the ATCC strain AF-102; ATCC-42202). This antigen is cytotoxic to mammalian cell lines and possesses ribonuclease activity.

Homogeneity of the purified 18 kD antigen was established on Sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) and high pressure liquid chromatography (HPLC).

Monoclonal antibodies raised against 18 kD allergen/antigen (Asp f1) (Moser et al, 1992 and Arruda et al, 1992) of an American type culture collection (ATCC) strain of *A. fumigatus* (AF-102; ATCC-42202) reacted with the 18 kD allergen/antigen of the present invention isolated from the *A. fumigatus* strain 285. The gene for 18 kD allergen/antigen was identified, sequenced and overexpressed. The deduced aminoacid sequence of the 18 kD allergen/antigen was analysed by ten different algorithmic programmes based on hydrophilicity, amphipathy, accessibility, mobility, antigenicity etc. This computerised analysis revealed presence of few sequences of T & B cell epitopic nature. Five of these probable epitopes were synthesised by solid phase method and studied in detail.

Synthesised epitopic sequences were characterised by Fast atom bombarding mass spectroscopy (FABMS) and High pressure liquid chromatography (HPLC) (Figures 7 to 16).--

---

Please replace the paragraph beginning on page 17, line 15, with the following rewritten paragraph:

A44 --Thus, this experiment confirms that a polyclonal or monoclonal antibody can be procured in the mouse which recognise the allergens/antigens of *A. fumigatus* or substructures (peptides) thereof. Such antibodies, in particular the monoclonal antibodies, can be used in antigen detection method like the sandwich ELISA for the detection of the *A. fumigatus* antigens in the human invasive aspergillosis specimens leading to immunodiagnosis of aspergillosis. --

Please replace the paragraph beginning on page 19, line 22, with the following rewritten paragraph:

A45 --4. The whole protein antigens need not be used for serodiagnosis. Small synthetic peptide epitopes facilitate fidelity thereby enhance reproducibility. Current peptides can replace the native antigen of *A. fumigatus* for diagnosis.--

Please replace the Sequence Information section beginning on page 22, line 4, with the following rewritten section:

--Sequence Information--

General Information

1. Sequence characteristics

A46 (A) Length: 17

(B) Type: Protein (INQQLNPKTNKWEDKRY), (SEQ ID NO: 1)

DNA ATC AAC CAA CAG CTG AAT CCC AAG ACA AAC AAA

TGG GAA GAC AAG CGG TAC (SEQ ID NO: 12)

cDNA TAG TTG GTT GTC GAC TTA GGG TTC TGT TTG TTT

ACC CTT CTG TTC GCC ATG (SEQ ID NO: 13)

RNA UAG UUG GUU GUC GAC UUA GGG UUC UGU UUG

UUU ACC CUU AUG UUC GCC AUG (SEQ ID NO: 14) --

2. Molecule type: Protein

3.Hypothetical: No

4.Antisense: No

5.Original source

(A) Organism: *Aspergillus fumigatus*

(B) Isolate: ATCC strain AF-102; ATCC-42202)

(C) Cell type: Fungus

6. Immediate source

(A) Library: No

(B) Clone: No

(C) Synthetic: Yes

7. Feature

Name/Key: Pep1, 17 aminoacids peptide

8.Identification method

(A) How you would identify: Aminoacid sequencing

(B) Other information : Binds specifically to *A. fumigatus* specific antibodies

Information for Sequence ID No. 2

1. Sequence characteristics

(A) Length: 11

(B) Type: Protein (LNPKTNKWEDK), (SEQ ID NO: 2)

DNA CTG AAT CCC AAG ACA AAC AAA TGG GAA GAC AAG (SEQ ID NO:15)

cDNA GAC TTA GGG TTC TGT TTG TTT ACC CTT CTG TTC (SEQ ID NO: 16)

RNA GAC UUA GGG UUC UGU UUG UUU ACC CUU AUG UUC (SEQ ID NO: 17)

(C) Standardness: FABMS-1373 amu--

2.Molecule type: Protein

3.Hypothetical: No

4.Antisense: No

5.Original source

A46  
cont

(A) Organism: *Aspergillus fumigatus*

(B) Isolate: ATCC strain AF-102; ATCC-42202)

(C) Cell type: Fungus

6. Immediate source

(A) Library: No

(B) Clone: No

(C) Synthetic: Yes

7. Feature

Name/Key: Pep2, 11 aminoacids peptide

8. Identification method

(A) How you would identify: Aminoacid sequencing

(B) Other information : Binds specifically to A.  
*fumigatus* specific antibodies

Information for sequence ID no.3

1. Sequence characteristics

(A) Length: 8

(B) Type: Protein (INQQLNPK), (SEQ ID NO: 3)

DNA ATC AAC CAA CAG CTG AAT CCC AAG (SEQ ID NO: 18)

cDNA TAG TTG GTT GTC GAC TTA GGG TTC (SEQ ID NO: 19)

RNA UAG UUG GUU GUC GAC UUA GGG UUC (SEQ ID NO: 20)

(C) Standardness: FABMS-954 amu

2. Molecule type: Protein

3. Hypothetical : No

4. Antisense: No

5. Original source

(A) Organism: *Aspergillus fumigatus*

(B) Isolate: ATCC strain AF-102; ATCC42202)

(C) Cell type: Fungus--

6. Immediate source

(A) Library: No

(B) Clone: No

A46  
cont

(C) Synthetic: Yes

7. Feature

Name/Key: Pep3, 8 aminoacids peptide

8. Identification method

(A) How you would identify: Aminoacid sequencing

(B) Other information : Binds specifically to A.

fumigatus specific antibodies

Information for sequence ID no. 4

1. Sequence characteristics

(A) Length:15

(B) Type: Protein (INQQLNPKTINKWEDK), (SEQ ID NO: 4)

DNA ATC AAC CAA CAG CTG AAT CCC AAG ACA AAC

AAA TGG GAA GAC AAG (SEQ ID NO:21)

cDNA TAG TTG GTT GTC GAC TTA GGG TTC TGT TTG

TTT ACC CTT CTG TTC (SEQ ID NO:22)

RNA UAG UUG GUU GUC GAC UUA GGG UUC UGU

UUG UUU ACC CUU AUG UUC (SEQ ID NO:23)

(C) Standardness: FABMS-1918 --

2.Molecule type: Protein

3.Hypothetical : No

4.Antisense: No

5.Original source

(A) Organism: *Aspergillus fumigatus*

(B) Isolate : ATCC strain AF-102; ATCC42202)

(C) Cell type: Fungus

6. Immediate source

(A) Library: No

A 46  
cont

(B) Clone: No

(C) Synthetic: Yes

7. Feature

Name/Key: Pep 4, 15 aminoacids peptide

8. Identification method

(A) How you would identify: Aminoacid sequencing

(B) Other information : Binds specifically to *A. fumigatus* specific antibodies

Information for sequence ID no.5

1.Sequence characteristics

(A) Length: 7

(B) Type: Protein (TNKWEDK), (SEQ ID NO: 5)

DNA ACA AAC AAA TGG GAA GAC AAG (SEQ ID NO: 24)

cDNA TGT TTG TTT ACC CTT CTG TTC (SEQ ID NO: 25)

RNA UGU UUG UUU ACC CUU AUG UUC (SEQ ID NO: 26)

(C) Standardness: FABMS - 919--

2.Molecule type: Protein

3.Hypothetical: No

4.Antisense: No

5.Original source

(A) Organism: *Aspergillus fumigatus*

(B) Isolate: ATCC strain AF-102; ATCC42202)

(C) Cell type: Fungus--

6. Immediate source

(A) Library: No

(B) Clone: No

(C) Synthetic: Yes

A46  
cont



7.Feature

Name/Key: Pep5, 7 aminoacids peptide

8. Identification method

(A) How you would identify: Aminoacid sequencing

(B) Other information : Binds specifically to *A. fumigatus* specific antibodies

Information for sequence ID no. 6

1.Sequence characteristics

(A) Length :13

(B) Type: Protein (LNPKTNKWEDKRY), (SEQ ID NO: 6)

DNA CTG AAT CCC AAG ACA AAC AAA TGG GAA GAC AAG CGG

TAC (SEQ ID NO: 27)

CDNA GAC TTA GGG TTC TGT TTG TTT ACC CTT CTG TTC GCC

ATG (SEQ ID NO: 28)

RNA GAC UUA GGG UUC UGU UUG UUU ACC CUU AUG UUC GCC

AUG (SEQ ID NO: 29)

(C) Standardness: FABMS-1835--

2.Molecule type: Protein

3.Hypothetical: No

4.Antisense: No

5.Original source

(A) Organism: *Aspergillus fumigatus*

(B) Isolate : ATCC strain AF-102; ATCC-42202)

(C) Cell type: Fungus

6.. Immediate source

(A) Library: No

(B) Clone: No

(C) Synthetic: Yes

7.Feature

Name/Key: Pep. 6, 13 aminoacids peptide

8.Identification method

A46  
cont